

## REMARKS

Claims 1 and 5-11 are pending.

Claim 1 is amended.

### **Claim 1 Amendment**

Claim 1 is amended to clarify that the microorganism is stored in a storage medium that comprises water and the growth medium in step ii) and that the nitrile is contacted with the microorganism in the storage medium of step iii) without recovery of the microorganism using centrifugation or filtration.

This amendment is supported by the disclosure on page 12, lines 16-20.

No new matter has been added.

### **35 USC 102(b)**

**Claims 1, 5-7, 9 and 11 are rejected under 35 USC 102(b) as being anticipated by US 5,705,382, Endo.**

Endo teaches a method for preserving a suspension of cells or immobilized cells under special conditions wherein cells are preserved/stored in a highly concentrated aqueous solution having a molarity ranging of from 100 mM to the saturation concentration of at least one inorganic salt.

The process of US'382 as a whole is described by the following:

- The microorganism is cultured, which is carried out "until the enzyme of interest reaches its maximum activity" (see col. 3, lines 50-55).
- The resulting cells are collected by centrifugation and washed with a buffer (see col. 3, lines 56-57).
- Cell suspension is mixed with the solution of inorganic salts (see col. 3, lines 58-60) or the washed cells are immobilized.
- The thus preserved cells are used in a production reaction as a conversion catalyst, wherein the cell suspension may be added to the reaction solution directly or after washing (col. 4, lines 31-34).

Clearly, a storing step follows a culturing step, but between the steps cells are separated, i.e. cells are separated before any further reaction with a nitrile. In contrast according to the present invention, cells are retained in the storage medium (growth medium) and are contacted with a nitrile to form an amide.

Hence, US'382 does not anticipate the claimed invention.

**Claims 1, 5-9 and 11 as amended are rejected under 35 USC 102(b) as being anticipated by US 5,089,411 (Yamada et al.).**

Yamada relates to a method of culturing the strain *Rhodococcus rhodochrous* J-1 in a culture medium comprising urea or a urea derivative (cf. claim 1), i.e. it is only directed to a culturing method. There is no disclosure of a method of storing a microorganism capable of producing a nitrile hydratase biocatalyst.

As described in col. 6, lines 5-6, the nitrile hydratase activity is measured with cells which are isolated from a culture fluid.

Thus, cells are separated after culturing from the growth medium. Contrary thereto, according to the present invention, cells are retained in the storage medium (growth medium) and are contacted with a nitrile as a substrate without using a separation step.

Hence, US'411 does not anticipate the claimed invention.

### **35 USC 103(a)**

**Claims 1-11 are rejected under 35 USC 103(a) as being unpatentable over US 5,705,382 (Endo et al.), US 5,089,411 (Yamada et al.), Nagasawa et al and US 6,395,467 (Fahy et al.).**

The claimed method clearly discloses that the inventive stored cells are not separated before contacting the nitrile to catalyze the reaction to the intended amide.

None of the cited references teach storing cells in the growth medium. None of the cited references teach conversion of the nitrile to an amide in the presence of the growth medium.

US'467 (Fahy et al.) relates to a method of preservation of biological material by contacting the material with a cryoprotectant solution, freezing said material in contact with the cryoprotectant solution, thawing and removing the cryoprotectant solution from the thawed biological material (cf. claims 11 and 1). The cryoprotectant solution may contain urea, but also polyvinyl alcohol. The cryoprotectant solution is clearly different from any growth medium for culturing microbes having a specific biocatalytic activity.

Thus, US'467 teaches the use of another medium for preserving cells and the removal of said medium prior to any further processing steps. Hence, US'467 cannot make up for the deficiencies of Endo or Yamada.

It is known that microbial cells can be stored at low temperature (4°C) in the same medium in which they were grown, but the enzyme activity tends to decrease in general after a time; and, before using said cells as a biocatalyst in any conversion step, the cells will usually be separated from the growth medium.

It has surprisingly been found that during the storage period the activity of the biocatalyst comprising nitrile hydratase activity actually increases (page 9, lines 29-30) which is also shown in Example 1 of the present application. This effect could not be predicted from prior art.

Therefore, the claimed invention is not rendered obvious by the cited references.

Reconsideration and withdrawal of the rejection of claims 1, 5-11 is respectfully solicited in light of the remarks and amendments *supra*.

Since there are no other grounds of objection or rejection, passage of this application to issue with claims 1, 5-11 is earnestly solicited.

Applicants submit that the present application is in condition for allowance. In the event that minor amendments will further prosecution, Applicants request that the examiner contact the undersigned representative.

Respectfully submitted,



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Enclosure: Petition for one (1) month extension of time.